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Underlying reasons of the controversy over adverse effects of Bt toxins on lady beetle and lacewing larvae

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Abstract

We outline important underlying reasons that fuel the decades-long controversy over adverse effects of Bt toxins expressed in genetically modified plants on beneficial, nontarget organisms. Inconsistent evaluation standards and asymmetrical levels of scrutiny applied to studies reporting significant adverse effects compared to those finding no adverse effects are described using the examples of the green lacewing (*Chrysoperla carnea*) and the two-spotted lady beetle (*Adalia bipunctata*). Additionally, the chosen style and concerted nature of the rather confrontational counter study and responses in the lady beetle cases bear striking similarities to other reported examples in the field of biosafety/risk science of genetically modified plants and to other fields of applied industrial techno-science that suggest deeper issues that go well beyond science. We call for a constructive and respectful scientific discourse where moving the frontiers of our collective knowledge forward takes center stage. Reported phenomena based on robust data must not be rejected or delegitimized on their being surprising and lacking an explained mechanism at the time of their discovery. Exploring mechanisms often requires entirely different expertise and methodologies than those of the discoverers. In particular, in biosafety/risk sciences, plurality of arguments and critical research approaches have to be embraced and actively encouraged rather than discredited or even silenced if we are to learn our 'late lessons' from past technology introductions.

In 2008/2009, Schmidt and colleagues [1] published a study reporting lethal effects of the microbial Bt toxins Cry1Ab and Cry3Bb on the biological control organisms *Adalia bipunctata*, a lady beetle. Based on this study and in concert with at least 30 other publications, Mon810 cultivation was banned in Germany in 2009. This policy response triggered two commentaries and one experimental study all published in the journal *Transgenic Research* that question the scientific basis of the German ban or claim to disprove the adverse effects of the Bt toxins on *A. bipunctata* reported by Schmidt and colleagues, respectively [2-4]. In a parallel paper, we report new data of a study that was undertaken to investigate the underlying reasons for the different outcomes and rebut some of the scientific aspects of the criticism voiced in the three publications. Here, we wish to take the opportunity to comment on some broader scientific aspects and issues that go beyond the experimental science delivered in the parallel paper. Given the strong language and the seemingly concerted effort we were confronted with in these three counter papers and, of course, in blogs and other fora, we find it justified, even necessary to, at least once, offer our evaluation and position on these criticisms.

In principle, we welcome the fact that our studies continue to stimulate debate and occasionally more research. However, it is unfortunate that the study by Alvarez-Alfageme et al. [2] was apparently set up primarily with the aim of disproving the results reported by Schmidt et al. [1]. Of course, any science has to withstand attempts at falsification just as any research result stands until new data emerges, pushing the frontiers of knowledge forward. However, the uncalled for, unnecessarily confrontational, and at times rather disrespectful unscientific nature of the language chosen obviously indicates deeper underlying issues. Other such confrontational responses and deliberate counter studies come to mind, as for example, the decades-long controversies on the risks connected with smoking, asbestos, or currently on-going

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bisphenol A and mobile telephones, where 'manufacturing uncertainty' has been well researched and documented, for example, by Michaels and Morforton [5]. We cannot know at present whether similar motivations drive the dogmatic 'refutations' which we find with respect to peer-reviewed results on potential harm from genetically modified organisms [GMOs], but reason alone forces one to wonder about this. More past examples of 'shooting the messenger' styles of responses following the publication of inconvenient research can also be found in the *Late Lessons from Early Warnings: The Precautionary Principle 1896-2000* published by the European Environment Agency [6], which in hindsight clearly generated a lot of unnecessary damage and avoidable human suffering. Indeed, here is a striking precedent of a previous case as well, as Rauschen [3] also notes in his opinion piece but for other reasons. Similar counter studies were launched to disprove the reporting of adverse effects of Bt toxins on another predatory species, the green lacewing (*Chrysoperla carnea*), roughly 10 years ago. We argue that, in addition to the mentioned argument, double standards and asymmetrical levels of scrutiny are being applied to studies reporting adverse effects of Bt toxins expressed in genetically modified [GM] crops on nontarget organisms that continue to fuel the debate. In the following paragraphs, these points are explained and supporting evidence is provided.

Double standards: debated routes of ingestion/exposure

The green lacewing, another important biological control organism, is also routinely tested for nontarget effects of Bt toxins for regulatory purposes in the approval process of Bt plants. The testing protocol follows closely the one used for pesticide testing which is based on the use of meal moth eggs that are coated with the test substance. Pesticides are externally applied on plants and insects and are often poisons that are taken up via skin or trachea (breathing apparatus) which is how they exert their effect. Bt toxins are gut poisons that must be ingested to unfold their effects. It is largely undisputed and has been pointed out for many years that the larvae of *C. carnea* are truly incapable of ingesting compounds deposited on the exterior of the eggs due to their very distinct, strictly piercing-sucking mouthparts (see Figure 1 for comparison of the mouthparts of *C. carnea* and *A. bipunctata*). We were among the first to point this out and express our concern about the inappropriateness of using coated meal moth eggs for testing Bt toxins [7].

To our knowledge, no comparable criticism has been published or voiced by the authors of the three papers discussed here regarding these obvious shortcomings in such trials that represent the prime data basis for safety assessments of Bt crops in the regulatory approval process. On the contrary, when we published our studies a decade ago revealing these shortcomings for green lacewing larvae and documenting that Bt toxins - both of microbial origin and of plant-origin - caused significant lethal effects in green lacewing larvae when administered directly or via prey into their gut using a protocol that ensured ingestion [7-9], they triggered a strikingly similar response involving some of the same authors of the Alvarez-Alfageme et al. paper [2]. Studies were published claiming to disprove the lethal effects of Bt toxins on *C. carnea* [10-12]. Also for this case, the differences in outcomes could be explained by the substantial differences in applied protocols and parameters measured [13], some of which bear resemblance to the protocols applied in this case of *A. bipunctata* (e.g., use of sugar solution and shorter exposure times). Rauschen [3] fails to acknowledge these pertinent issues when making reference to the green lacewing case. It is, however, quite interesting to note that the realization of the shortcomings we have been pointing out has at least been trickling into the regulatory requirements of the US Environmental Protection Agency [EPA]. This scientific deficiency is recognized in rulings like the following statement from 2007: 'In addition, it is questionable whether the green lacewings are ingesting the CryBb1 protein that is coated around moth eggs in a diet. Since green lacewings have piercing-sucking mouthparts, they may not be exposed to the protein on the external surface of the egg diet' [14]. However, instead of recommending tests with altered protocols that ensure ingestion of the Bt toxin, EPA recommends to drop this testing organism altogether and use instead another predator of the genus *Orius* [14] that has already been demonstrated not to be sensitive to Bt toxins (e.g., [15]). In addition, regarding lady beetles that are also routinely tested in safety trials for the regulatory approval of Bt crops, these were and still are often tested during the adult stage, which is inappropriate. It is undisputed that Bt toxins are gut toxins that exert their maximum effect during the juvenile stages of an insect. This shortcoming has also been recognized only years after the first commercial approvals by the EPA: 'EPA also believes, however, that lady beetle larvae would potentially have a higher risk of exposure to Cry2Ab2 than adults. Therefore, a dietary toxicity study will be required to determine the no observed effect concentration for lady beetle larvae... EPA has not previously required such a lady beetle larvae study for other registered PIP products...' [16]. For more details on these and other shortcomings in testing procedures for regulatory biosafety purposes of GM crops, we refer to an extended review by Dolezel et al. [17].

None of these obvious and recognized deficiencies led the authors of the three papers discussed here [1,3,4], nor the European Food Safety Agency [EFSA] for that matter, to apply a similar level of critical scrutiny as

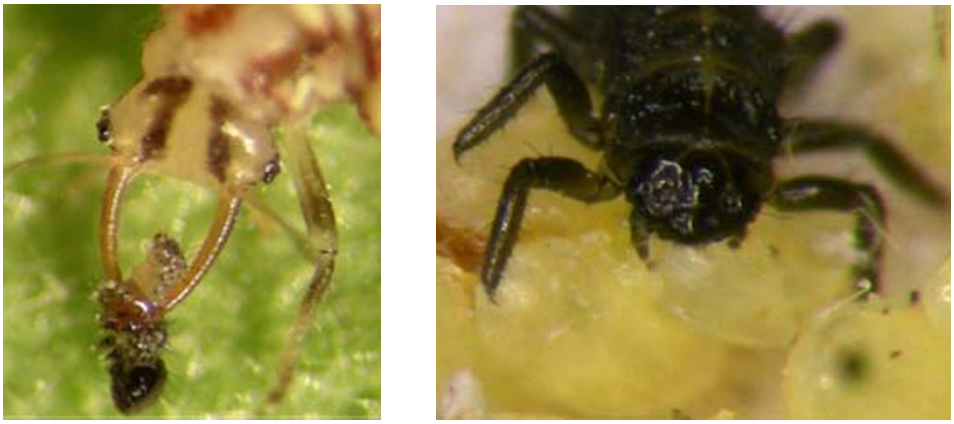

Figure 1 Pictures of mouth parts of first instar larvae. (**a**) *Chrysoperla carnea* (strictly piercing-sucking) and (**b**) *Adalia bipunctata* (biting, licking).

they have applied to Schmidt et al. [1] and other studies reporting adverse effects, when they deal with these regulatory biosafety studies that constitute the core data basis for regulatory approvals.

Level of scrutiny

The above-described double standards reinforce the longstanding observation that in the GMO environmental risk assessment field, often only those studies that report adverse effects are subjected to 'extra' scrutiny by the regulatory science community and some scientist circles including the authors of the three papers discussed here. This practice has also been applied by EFSA and was confirmed in an interview with a former EFSA GMO panel member who stated: 'Of course, studies that describe potential negative environmental effects of GMOs are discussed particularly intensively' [18]. According to Millstone et al. [19], this practice is interpreted by the European public as an illegitimate support for the biotechnology industry, by the risk assessor. They state that 'greater institutional care was taken to try to avoid false positives than to avoid false negatives. That implies that critical scrutiny has been applied in an asymmetrical fashion that *prima facie* seems difficult to reconcile with a precautionary approach' [19]. The European Commission does indeed claim to follow the precautionary principle in all its regulatory appraisal processes.

Choice of language and displayed attitude

Lastly, we cannot help but also notice the striking resemblance of the three concerted papers discussed here to the strategy extensively documented and described by Waltz [20] in her *Nature* article and summarized as follows: 'Papers suggesting that biotech crops might harm the environment attract a hail of abuse from other scientists. Behind the attacks are scientists who are determined to prevent papers they deem to have scientific flaws from influencing policy-makers. When a paper comes out in which they see problems, they react quickly, criticize the work in public forums, write rebuttal letters, and send them to policy-makers, funding agencies and journal editors.' We also find confirmation for the observation of an editor of the scientific journal *Environmental Entomology* after having been subjected to a similar ordeal with another 'problem paper' who states that 'part of what exasperates me is that they (the attacking scientists) have declared themselves to be the experts in this field and forcefully present themselves as the ultimate arbiters of truth' [20]. There is ample evidence for such a self-acclaimed 'arbiters of truth' attitude displayed 'forcefully' in all three publications [1,3,4]. As we find in particular those by Alvarez-Alfageme et al. [1] and Rauschen [3] unscientific, disrespectful, and thus, unacceptable for a proper scientific discourse, we do not repeat them here but refer to the original sources which also reflect on their preferred chosen outlet, the journal *Transgenic Research*. Needless to say that none of the attacked authors of the Schmidt et al. [1] study were informed prior to the publication of the three papers let alone offered the opportunity to defend and/or clarify their research, in response. This is one way a debate can be conducted but clearly not a constructive nor a properly scientific one at all. We hope that with both the scientific content of our follow-up study and the tone of this commentary, we have succeeded in setting a higher standard for scientific disagreement that aims to generate more and better knowledge, whichever way it may point in policy terms, rather than a more confrontational dogma. Better standards also include that at some point, one has

to stand up to those who are not adhering to a common proper code of conduct and to reveal exactly that.

Scientists concerned with the environmental risks of novel technologies ought not to dismiss new knowledge/phenomena and delegitimize unexpected results based on their being 'surprising' and lacking an explanatory mechanism at the time of discovery, or possibly triggering policy responses one happens to disagree with. Scientific progress often comes about by first discovering and reporting an unexpected and inexplicable novel phenomenon. Only if that phenomenon is deemed of sufficient curiosity and importance do we embark on exploring the underlying mechanisms. More often than not, this requires entirely different expertise and techniques than those of the discoverers. It is indisputable that the lack of an explanatory mechanism does not - or rather, should not - invalidate an empirically established phenomenon. We have repeatedly established the phenomenon that Bt toxins can induce a significantly higher mortality in the two nontarget beneficial insects, green lacewings and the lady beetle *A. bipunctata*, with scientific data which are among the statistically and methodologically most robust that are published on this issue in the scientific literature to date (see the parallel paper to this commentary and Hilbeck and Schmidt [13] for extensive comparison of the statistical values and experimental methodologies). It is time to move beyond this dogmatic denial and 'killing the messenger' stage of the debate. Researchers with other expertise are now called upon to engage in research that genuinely aims at studying and elucidating the mechanisms of this established phenomenon and push forward our knowledge on modes of action of Bt toxins and modes of interaction (e.g., [21]) with other cofactors (e.g., [22]). After all, an increasing number of so-called 'stacked' Bt plants are reaching our fields and markets today that contain up to six Bt toxins, and hardly any of which have been tested for their combinatorial effects due to the 'lack of a known mechanism'. We find this a non-precautionary and insufficient scientific reason for simply foregoing the expenditure of testing, and perhaps even more importantly, possibly finding potential adverse effects, and thus, being able to prevent them.

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Authors' contributions
AH wrote the commentary. AH, together with MM and MT and along with the inspiration from numerous colleagues far beyond the circle of authors of this and the parallel science article, discussed and developed the arguments. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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